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Role of native soil biology in Brassicaceous seed meal-induced weed suppression

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ABSTRACT

Biologically based weed control strategies are needed in organic and low-input systems. One promising practice is the application of Brassicaceous seed meal (BSM) residue, a byproduct of biodiesel production. When applied as a soil amendment, BSM residue has exhibited potential bioherbicide activity. In this study, tree fruit orchard soils were treated with various BSMs and the impact of *Pythium* on weed suppression was examined in field and greenhouse studies. Although weed control obtained in response to Brassicaceous residue amendments has been repeatedly attributed solely to release of allelopathic phytochemicals, multiple lines of evidence acquired in these studies indicate the involvement of a microbiological component. Reduced weed emergence and increased weed seedling mortality were not related to BSM glucosinolate content but were correlated with significant increases in resident populations of *Pythium* spp. in three different orchard soils. Seed meal of *Brassica juncea* did not amplify resident *Pythium* populations and did not suppress weed emergence. Application of *Glycine max* SM did stimulate *Pythium* spp. populations and likewise suppressed weed emergence. Application of a mefenoxam drench to *Pythium*-enriched soil significantly reduced weed suppression. These studies indicate that a microbial mechanism is involved in SM-induced weed suppression and that selective enhancement of resident pathogenic *Pythium* spp. can be utilized for the purpose of weed control.

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1. Introduction

Growth in organic and sustainable agricultural production systems has generated demand for compatible weed control strategies. Brassicaceous seed meal (BSM) residue, a waste product of the oil extraction process, can provide a local resource for supplemental nutrients (Hoagland et al., 2007), disease control (Lazzeri and Manici, 2001; Mazzola et al., 2001; Zasada and Ferris, 2004; Mazzola and Mullinix, 2005), and/or weed suppression (Brown and Morra, 1997). However, the mechanisms contributing to the observed BSM weed control remain unclear (Boydston and Hang, 1995; Brown and Morra, 1997).

Decreased weed emergence has been repeatedly documented following soil incorporation of Brassicaceous crop and BSM residues (Boydston and Hang, 1995; Al Khatib et al., 1997; Brown and Morra, 1997). The mechanism of weed suppression has been attributed to allelopathy, which is defined as the inhibitory effect of one plant or microorganism on another through chemical release from the donor to the environment (Kobayashi, 2004). Glucosinolate hydrolysis products are thought to be responsible for the weed suppression induced by Brassicaceous residues (Brown and Morra, 1997). The hydrolytic enzyme, myrosinase, and water are required for glucosinolate hydrolysis. The type, concentration, and functionality of glucoinolate hydrolysis products vary among Brassicaceous species. Glucosinolates are present in all Brassicaceous plant parts, but are most concentrated in seed (Borek and Morra, 2005). If cold pressed, residual BSM retains glucosinolate content and viable myrosinase after seed oil extraction (Borek and Morra, 2005). Therefore, it is reasonable to hypothesize that glucosinolate hydrolysis products have a role in the weed suppression resulting from application of BSM.

Although weed suppression by Brassicaceous residues has long been attributed to glucosinolate induced allelopathy, there has not been a consistent relationship between observed weed suppression and measured glucosinolate content. For example, significant plant suppression has been observed with low glucosinolate content *Brassica napus* residues (Boydston and Hang, 1995; Brown and Morra, 1996; Al Khatib et al., 1997). These authors suggested either effective action by a relatively small amount of a specific but unidentified glucosinolate hydrolysis product, or that microbial degradation resulted in production of other inhibitory compounds. Some glucosinolate hydrolysis products such as ionic thiocyanate have biocidal effects, and are used as the active ingredient in several commercial

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herbicides (Borek and Morra, 2005). However, these products control weeds at effective ionic thiocyanate (SCN^-) concentrations of 137–1366 kg SCN^- ha^{-1}, much higher than that found in BSM amendment rates that have been found to be phytotoxic (Borek and Morra, 2005). Phytotoxicity has been observed at BSM amendment rates of 1000–4000 kg SM ha^{-1}, with only 8.8–35.3 kg SCN^- ha^{-1}, assuming complete conversion to toxic hydrolysis products (Borek and Morra, 2005). In addition, soil physical, chemical, and biological characteristics influence expression and longevity of allelochemicals under field conditions (Inderjit et al., 2001).

Incorporation of plant residue, including *Brassica* spp., is also commonly associated with rapid increases in total microbial activity, which can include plant pathogenic soil fungi and oomycetes (Grünwald et al., 2000; Manici et al., 2004; Cohen et al., 2005), with many capable of inciting root, stem, or seed rots (Pitty et al., 1987) that can be fatal to both crop and weed species. Many members of the genus *Pythium* incite both pre- and post-emergent damping-off of plants. Populations of *Pythium* spp. in soil are amplified in response to organic matter addition, survive in competition with other microorganisms (Chen et al., 1988) and withstand frequent cultivation (Grünwald et al., 2000; Mazzola and Gu, 2000).

Application of Brassicaceous amendments may provide an alternative weed control strategy, but the mechanism of action must be better understood to generate guidelines and recommendations for use of this practice as a management tool. These studies were performed in or with multiple orchard soils to test the hypothesis that induced amplification of resident *Pythium* spp. contributes to the weed suppression observed in response to BSM amendments.

2. Materials and methods

2.1. Soils and soil treatments

Studies were conducted at or in soils collected from three experimental orchards: the Columbia View Experimental (CV) orchard, Orondo, WA; the Wenatchee Valley College-Auvil Teaching and Demonstration (WVC) orchard, East Wenatchee, WA; and the Tukey Horticulture Research and Experimental (TU) orchard, Pullman, WA. Soils at these sites are characterized as Adkins very fine sandy loam (coarse–loamy, mixed, mesic Xeric Haplocalcid) with 1.3% organic matter (OM) and pH 7.6, Pogue sandy loam (coarse–loamy over sandy or sandy–skeletal, mixed, mesic Aridic Haploxeroll) with 1–2% OM and pH 6.1–7.3, and Thatuna silt loam (fine-silty, mixed, mesic Oxyaquic Argixeroll) with 4–5% OM and pH 6.8, respectively. Plots at WVC and TU orchards are under organic management.

Amendments used in field and greenhouse studies included a low glucosinolate (glucosinolate content (GLC) = 21.8 μmol g^{-1}) commercial rapeseed, *B. napus* cv. Dwarf Essex (Montana Specialty Mills, Great Fall, MT), and two high glucosinolate mustard varieties, *Brassica juncea* cv. Pacific Gold (GLC = 303 μmol g^{-1}) (Brown et al., 2004), and *Sinapis alba* cv. Ida Gold (GLC = 244 μmol g^{-1}) (Brown et al., 1997). Nitrogen contents of the BSMs were 5.57, 6.09, and 6.84%, respectively (Mazzola et al., 2007). Greenhouse experiments also included a non-glucosinolate containing soybean (*G. max*) seed meal (no glucosinolate, 3% N) treatment and a pasteurized soil treatment. All amendments were applied to soil at a rate of 0.3% vol/vol. All field and greenhouse experiments included a non-treated control. In 2005, the field experiment carried out at CV orchard included a 1,3-dichloropropene-chloropicrin (TeloneC17; DowElanco, Indianapolis, IN) soil fumigation treatment at 282 l ha^{-1}. A mefenoxam (Ridomil Gold EC 49% ai; Syngenta, Greensboro, NC) soil drench was used in the 2006 field experiment and all greenhouse experiments to selectively reduce plant infection by *Pythium* spp.

2.2. Greenhouse experiments

Composite soil samples were collected at WVC in spring 2005 (WVC1), autumn 2005 (WVC2), and at TU orchards in spring 2006 for use in greenhouse assays. Soil was also collected in spring 2006 from an experimental plot at CV orchard and an area immediately adjacent with native (uncultivated) shrub steppe vegetation. Ten soil samples were collected from within the root zone of random trees in established orchard sites to a depth of 10–30 cm, approximately 1–2 m from the tree base and pooled. Soil was stored at ambient greenhouse conditions until experiments were initiated. Three replicate soil samples from each site/date were pooled and stored at 4 °C for subsequent laboratory analysis. For each experiment, soil was premixed using a cement mixer and 2.5 l aliquots of soil were placed in 5-l tubs. Seed meal amendments were applied to soil in two tubs per treatment, hand mixed and covered with lids during a 4 d incubation in the greenhouse at 22 ± 4 °C. At completion of the incubation period, a composite soil sample was collected from each treatment for laboratory analysis. At the same time, mefenoxam was diluted to 0.635 ml l^{-1} and 116.7 ml was applied to soil in one of the tubs representing each treatment. Soil from each tub was then placed in conical tubes (21 × 4 cm). Prior to planting, germination rates for each plant spp. were determined by placing 20 seeds onto moistened filter paper in a petri dish, and counted after 48 h (Mazzola and Cook, 1991). Subsequently, five *Triticum aestivum* (Wheat cv. Madsen) seeds, 10 *Vicia villosa* (Hairy Vetch) seeds, 10 *Amaranthus retroflexus* (Pigweed) seeds, or seven *Echinochloa crusgalli* (Barnyardgrass) seeds were planted into conical tubes. Each seed type × soil treatment combination was replicated in 10 growth tubes. Plants were individually watered when a dry soil surface was observed. Plant emergence was recorded at 5 d and again at harvest 21 d after planting. Twelve days after planting, three cones per seed type/soil treatment were randomly selected for determination of *Pythium* soil populations and root infection.

2.3. Experimental field plots

Field plots, 3.05 m^2, were established at CV orchard in spring 2005 and 2006 in a randomized complete block design with split-plots and five replicates. Seed meal amendments were applied at 8533 kg ha^{-1}, and incorporated to 15 cm depth using a rotovator. Forty-eight hours after BSM amendment, mefenoxam (0.635 ml l^{-1}) aqueous solution was applied to half of each plot at 1.48 ml m^{-2}. In 2005, BSM was applied on 21 April and half of each split-plot was split again and covered with a 152-μm thick clear plastic sheet (Sunbelt Plastics, Monroe, LA), which was removed on 23 May (32 d). Plastic was not applied to plots in 2006.

In 2005, approximately 90 d after seed meal application, all shoot and root biomass was collected from each plot at CV orchard and divided into grass and broadleaf species. At the same site, aboveground weed biomass was also collected from a newly established orchard planting employing the same soil treatments using the same method. In 2006, 3 d following SM amendment, five *T. aestivum* seeds (cv. Madsen) were planted into each split-plot and germination was recorded after 14 d. Forty days after amendment application, four sub-samples (0.1 m^2 each) of aboveground weed biomass were cut and pooled for analyses within each split-plot. All plant samples were oven-dried at 50 °C for 48 h and weighed to determine dry biomass.

Soil samples were collected at 0, 3, 8 and 15 d post-BSM amendment. Three or four sub-samples were collected using a 2-cm diameter soil probe and pooled for analyses. Sampling depth of 0–10 or 10–30 cm is indicated on all data in results. All soil samples were stored at 4 °C until analysis.

2.4. Characterization of soil and plant colonizing Pythium populations

Three separate 5-g soil sub-samples from each field or greenhouse treatment were suspended in 25 ml sterile distilled water, vortexed 60 s and serial dilutions were plated on a *Pythium* semi-selective growth medium (PSSM; Mazzola et al., 2001). After 48 h, adhering soil was washed from plates under running water, and colonies exhibiting typical *Pythium* morphology were enumerated. Hyphal plugs from representative *Pythium* colonies from each plate were transferred to new plates.

In greenhouse assays, composition of the *Pythium* population recovered from plant tissues was determined. Plants from each growth tube were individually removed, rinsed with tap water, and six root segments 3 cm in length were plated onto PSSM. In the pots where no plants emerged, large weed seeds (*T. aestivum* and *V. villosa*) were extracted from the pot and plated onto PSSM. *Pythium* infection of each root/seed was recorded after 48 h.

Initial species identifications of *Pythium* isolates recovered were determined by DNA sequence analysis. Three 0.4 cm diameter plugs were excised from the growing margin of individual cultures, transferred to 5 ml 20%-strength potato dextrose broth, and incubated on rotating platform (150 rpm) at ambient laboratory conditions. DNA was extracted from *Pythium* mycelium using a MoBIO Ultraclean Soil DNA kit (Carlsbad, CA), and stored at −20 °C until analysis. Polymerase chain reaction amplification of *Pythium* DNA was conducted using the primer set internal transcribed spacer (ITS) 4 and ITS5 (White et al., 1990) in a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA) using conditions previously described (Tewoldemedhin et al., 2006). Amplification products were confirmed by visual comparison to a 100 bp ladder following electrophoresis on a 1.5% agarose gel stained with ethidium bromide. Resulting amplicons were directly sequenced using a Dye Terminator Cycle Sequencing Quick Start Kit and a CEQ 8000 Genetic Analysis System capillary-based DNA sequencer (Beckman Coulter, Fullerton, CA) with ITS1 (White et al., 1990) as the sequencing primer. Sequences obtained were compared with the online NCBI BLAST database.

Restriction fragment length polymorphism (RFLP) analysis was also employed to characterize the composition of *Pythium* spp. populations. ITS amplicons generated from each *Pythium* isolate were digested individually in single enzyme reactions using HaeIII, HpaI, RsaI or TaqI. Each reaction contained 8 μl PCR product, 1 μl restriction enzyme, and 1 μl of the appropriate 10× digestion buffer. All digests were incubated at ambient conditions overnight except TaqI, which was incubated overnight at 65 °C. Digest patterns for each *Pythium* isolate were visualized by comparison to a 100 bp ladder following electrophoresis on a 1.5% agarose gel stained with ethidium bromide. Restriction patterns were compared to a library of RFLP patterns generated from representative *Pythium* isolates, which had been identified by sequence analysis and morphological characterization in this and previous studies (Mazzola et al., 2002).

2.5. Quantification of soil Pythium populations by real-time PCR

Pythium spp. in soils were quantified by real-time PCR (Schroeder et al., 2006). Briefly, DNA was extracted from soil using a MoBIO Ultraclean Soil DNA Isolation kit, from two 0.5 g soil samples per treatment. The DNA was employed in individual 20 μl reactions, conducted in duplicate using FastStart DNA Master SYBR Green I and a Roche Light Cycler, with conditions and primer pairs designed to amplify one of 10 *Pythium* spp. (Schroeder et al., 2006). After initial analyses, *P. paroecandrum*, *P.* aff. *echinulatum*, *P. irregulare* Group I, *P. ultimum*, *P. heterothallicum*, and *P. attrantheridium* primers were selected for use on all soil treatments.

Table 1a
Effects of seed meal amendments on percent emergence of *Triticum aestivum* when established in two orchard soils

Treatment	WVC1[a]	WVC2		TU	
	5 d	5 d	21 d	5 d	21 d
Control	62 a[b]	62 c	62 bc	20 e	32 d
Control + mefenoxam	82 ab	82 ab	90 ab	94 a	98 a
Pasteurized	94 a	94 a	96 a	96 a	92 a
Pasteurized + mefenoxam	88 a	88 a	86 ab	64 cd	72 de
B. napus	8 e	8 d	70 abc	4 f	18 de
B. napus + mefenoxam	92 a	92 a	80 abc	92 ab	96 a
B. juncea	68 bc	68 bc	64 bc	54 d	62 c
B. juncea + mefenoxam	90 a	90 a	78 abc	78 cd	84 ab
S. alba	30 d	12 d	20 d	4 f	6 e
S. alba + mefenoxam	90 a	90 a	70 abc	94 a	94 a
G. max	12 e	12 d	54 c	2 f	8 e
G. max + mefenoxam	94 a	94 a	74 abc	88 ab	86 ab

[a] Orchard designations: WVC, Wenatchee Valley College Orchard, East Wenatchee, WA, and Tukey, Tukey Horticulture Research and Experimental Orchard, Pullman, WA. WVC1 represents soil collected in spring 2005, and WVC2 represents soil collected in autumn 2005.
[b] Means in the same column followed by the same letter are not significantly different ($P > 0.05$; $n = 10$).

2.6. Statistical analysis

All statistical analyses were conducted with SAS 9.1 software (SAS Institute Inc., Cary, North Carolina). Data were subjected to analysis of variance and mean separation was based on Fisher Protected LSD. Results were considered significant at $P \leq 0.05$.

3. Results

3.1. Weed emergence and biomass in the greenhouse

Seed meal treatments resulted in significant ($P < 0.05$) reductions or increases in plant emergence, with the response being seed meal or plant dependent. *T. aestivum* emergence and survival were reduced by amendment of soil with *B. napus*, *G. max* or *S. alba* SM, relative to the control (Table 1a). In contrast, pasteurization, *B. juncea* amendment, and mefenoxam treatments typically increased plant emergence (Table 1a). Emergence of *V. villosa* was low overall and consistent treatment effects were not observed, although *V. villosa* emergence exhibited trends similar to those of *T. aestivum* in response to soil treatments (Tables 1a and 1b). Amendment with *S.*

Table 1b
Effects of seed meal amendments on percent emergence of *Vicia villosa* when established in two orchard soils

Treatment	WVC1[a]	WVC2		TU	
	5 d	5 d	21 d	5 d	21 d
Control	13 bcd[b]	13 bcd	30 c	12 c	22 d
Control + mefenoxam	23 ab	23 ab	49 a	23 abc	38 bcd
Pasteurized	10 cd	10 cd	47 ab	32 ab	64 a
Pasteurized + mefenoxam	20 abc	20 abc	45 ab	17 bc	49 ab
B. napus	11 cd	11 cd	41 b	18 abc	37 bcd
B. napus + mefenoxam	20 abc	20 abc	48 ab	22 abc	38 bcd
B. juncea	20 abc	20 abc	18 d	11 c	26 d
B. juncea + mefenoxam	25 a	25 a	45 ab	20 abc	40 bc
S. alba	11 cd	11 cd	4 e	13 c	29 cd
S. alba + mefenoxam	23 ab	23 ab	46 ab	28 abc	43 bc
G. max	5 d	5 d	13 d	17 bc	29 cd
G. max + mefenoxam	17 abc	17 abc	47 ab	33 a	41 bc

[a] Orchard designations: WVC, Wenatchee Valley College Orchard, East Wenatchee, WA, and Tukey, Tukey Horticulture Research and Experimental Orchard, Pullman, WA. WVC1 represents soil collected in spring 2005, and WVC2 represents soil collected in autumn 2005.
[b] Means in the same column followed by the same letter are not significantly different ($P > 0.05$; $n = 10$).

Table 1c
Effects of seed meal amendments on percent emergence of *Echinochloa crusgalli* when established in two orchard soils

Treatment	WVC1[a]	WVC2		TU	
	5 d	5 d	21 d	5 d	21 d
Control	48 bc[b]	48 bc	38 cd	27 c	28 ef
Control + mefenoxam	53 abc	53 bc	54 ab	43 ab	43 abcd
Pasteurized	56 ab	56 ab	43 bcd	49 a	54 a
Pasteurized + mefenoxam	56 ab	56 ab	53 ab	44 ab	47 abc
B. napus	51 abc	51 bc	54 ab	41 ab	33 de
B. napus + mefenoxam	44 c	44 c	61 a	42 ab	46 abcd
B. juncea	65 a	65 a	33 de	33 bc	33 cde
B. juncea + mefenoxam	55 ab	55 ab	53 ab	48 a	40 bcde
S. alba	31 d	31 d	19 f	33 bc	37 bcde
S. alba + mefenoxam	44 c	44 c	46 bc	44 a	51 ab
G. max	57 ab	57 ab	24 ef	23 c	16 f
G. max + mefenoxam	52 abc	52 bc	47 bc	44 ab	38 bcde

[a] Orchard designations: WVC, Wenatchee Valley College Orchard, East Wenatchee, WA, and Tukey, Tukey Horticulture Research and Experimental Orchard, Pullman, WA. WVC1 represents soil collected in spring 2005, and WVC2 represents soil collected in autumn 2005.
[b] Means in the same column followed by the same letter are not significantly different ($P > 0.05$; $n = 10$).

alba SM reduced emergence of *E. crusgalli* compared to the control in WVC soils. *G. max* SM amendment reduced *E. crusgalli* emergence only one time in WVC soils. The majority of pasteurization and mefenoxam treatments, and certain *B. juncea* amendments, increased *E. crusgalli* emergence relative to the control (Table 1c). Soil amendment with *S. alba* or *G. max* SM reduced *A. retroflexus* emergence in most cases, with most other treatments increasing *A. retroflexus* emergence (Table 1d). Biomass followed similar trends to emergence data for all species (not shown).

3.2. Weed emergence and biomass in the field

Soil treatment resulted in statistically significant ($P < 0.05$) reduction or increase in weed biomass and *T. aestivum* emergence, with the response being dependent on seed meal and plastic covering. In the new apple orchard planting established in 2005, *B. napus* SM amendment resulted in greater yield of grass biomass in comparison to all treatments except fumigation (Fig. 1). In separate plots established at CV in 2005, broadleaf weed biomass was reduced in all BSM-amended plots covered with plastic relative to uncovered plots (Fig. 2). In contrast, without plastic cover, biomass

Table 1d
Effects of seed meal amendments on percent emergence of *Amaranthus retroflexus* when established in two orchard soils

Treatment	WVC1[a]	WVC2		TU	
	5 d	5 d	21 d	5 d	21 d
Control	26 ab[b]	30 ab	20 de	26 ab	24 cdef
Control + mefenoxam	24 abc	29 abc	29 abcd	24 abc	36 abcde
Pasteurized	21 abc	33 abc	43 a	21 a	53 ab
Pasteurized + mefenoxam	26 ab	24 ab	33 abcd	26 abcd	37 abcd
B. napus	16 bcd	11 bcd	14 bcd	16 bcd	31 cdef
B. napus + mefenoxam	23 abc	43 abc	44 cde	23 a	27 abc
B. juncea	23 abc	26 abc	16 ef	23 abc	13 def
B. juncea + mefenoxam	37 a	29 abc	31 ab	37 abc	47 abc
S. alba	3 d	4 d	6 f	3 d	7 f
S. alba + mefenoxam	34 a	30 a	31 ab	34 ab	43 abcd
G. max	7 d	10 cd	10 ef	7 cd	13 ef
G. max + mefenoxam	23 abc	26 abc	24 cd	23 abc	30 bcdef

[a] Orchard designations: WVC, Wenatchee Valley College Orchard, East Wenatchee, WA, and Tukey, Tukey Horticulture Research and Experimental Orchard, Pullman, WA. WVC1 represents soil collected in spring 2005, and WVC2 represents soil collected in autumn 2005.
[b] Means in the same column followed by the same letter are not significantly different ($P > 0.05$; $n = 10$).

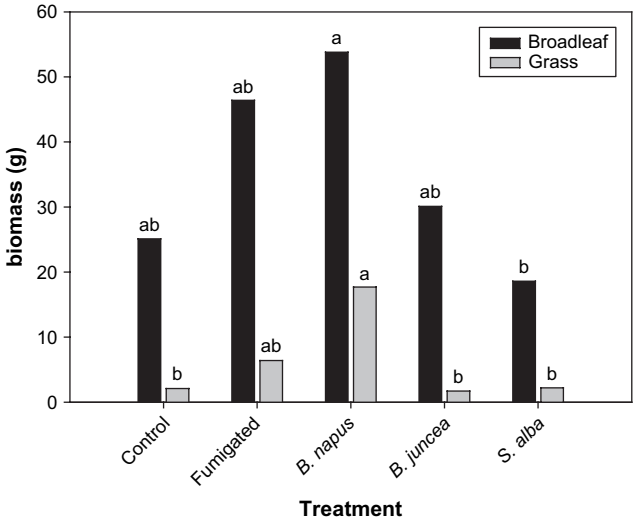

Fig. 1. Effect of Brassicaceous seed meal amendments on aboveground weed biomass in an apple planting established in 2005 at the Columbia View Orchard. Values, represented by bars, designated with the same letter are not significantly different ($P > 0.05$; $n = 5$).

from *S. alba*-amended plots was still lower than the non-treated control, while biomass from *B. napus*- and *B. juncea*-amended plots was greater than their respective plastic covered plots and control (Fig. 2). Grass biomass followed a similar trend but with no statistically significant differences (Fig. 2). In 2006, though trends were similar to those observed in 2005, there were no differences in weed biomass production among treatments (data not shown). However, emergence of planted wheat seeds was reduced in both *B. napus* and *S. alba* SM-treated plots relative to the control (Fig. 3); mefenoxam treatment of SM-amended plots eliminated the suppression of wheat emergence.

3.3. Pythium *soil populations*

In greenhouse experiments all soils exhibited significant ($P < 0.05$) increases in *Pythium* populations in response to *B. napus*, *S. alba*, and *G. max* SM, with the exception of CV-native soil (Table 2). Resident *Pythium* spp. were not detected in initial samples of CV-native soil, and SM amendments did not elicit a response in the

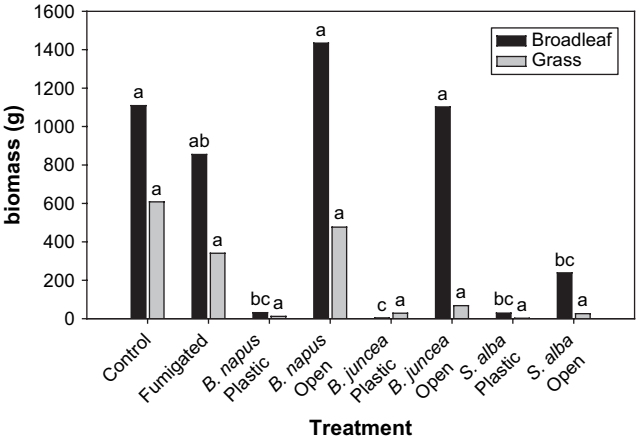

Fig. 2. Effect of Brassicaceous seed meal amendments on above and belowground weed biomass in 2005 at Columbia View orchard in field plots not planted to apple. Values, represented by bars, designated with the same letter are not significantly different ($P > 0.05$; $n = 5$).

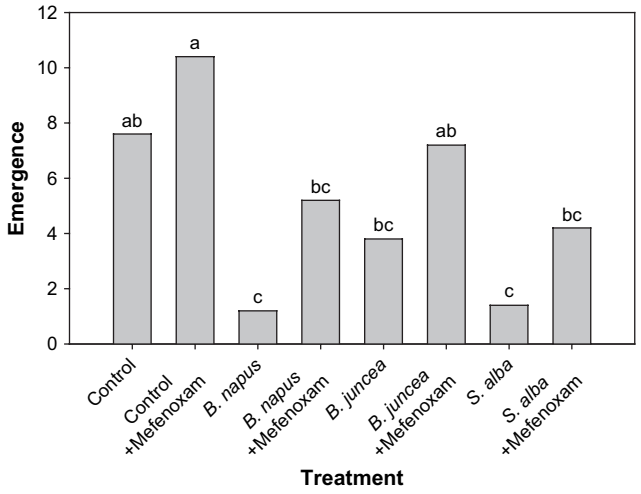

Fig. 3. Effect of Brassicaceous seed meal amendments on emergence of *Triticum aestivum* seeded in 2006 at Columbia View orchard in field plots not planted to apple. Values, represented by bars, designated with the same letter are not significantly different ($P > 0.05$; $n = 5$).

Pythium spp. population in this soil (Table 2). *Pythium* spp. populations reached similar densities after *B. napus*, *S. alba*, or *G. max* SM amendment, 1216–1916 cfu g^{-1}, in all orchard soils tested, but relative increases were much lower in TU orchard soil (Table 2). In all soils, *B. juncea* amendment resulted in a reduction of *Pythium* spp. numbers to near the limit of detection.

In field studies conducted at CV orchard, *B. napus* SM amendment, regardless of tarping, significantly ($P < 0.05$) elevated soil populations of *Pythium* spp. relative to the control in both 2005 (Table 3) and 2006 (not shown). In contrast, *Pythium* spp. numbers in *B. juncea*-amended plots were reduced to near zero. Time series data from 2006 revealed an initial *Pythium* decrease in all BSM-amended plots, followed by rapid increases in *B. napus*- and *S. alba* SM-amended soils, with populations reaching their highest in *B. napus*-amended plots (not shown). For all soil treatments, *Pythium* populations peaked approximately 8 d post-amendment and then declined.

3.4. Pythium *root and seed infection*

Recovery of *Pythium* spp. from roots and seeds of all plant types established in WVC2 and TU orchard soils amended with *S. alba* was significantly ($P < 0.05$) greater than the control and pasteurized treatments as well as the respective SM-amended soils treated with mefenoxam (Table 4). Similar results were found in all but one case with *G. max* SM-amended soil. In *B. napus* SM-amended soil, plant infection by *Pythium* spp. increased in five of eight analyses (Table 4). There was no difference between recovery of *Pythium* from roots and seeds in *B. juncea* SM-amended soil and control or pasteurized treatments. Seed and root samples from TU-amended soils were infected by *P. ultimum*, *P. attrantheridium* and *P. heterothallicum*, whereas plant tissues established in WVC2-amended soils were infected primarily by *P. irregulare*, and *P. ultimum*. There was no preference for a particular *Pythium* spp. to infect one plant species over another.

Table 2
Effect of seed meal amendments on populations of *Pythium* spp. (cfu g^{-1} soil) recovered from three different orchard soils in greenhouse experiments

	WVC1[a]	WVC2	TU	CV	CV-native
Initial	150 c[b]	150 c	616 cd	150 d	0 a
Control	33 c	67 c	833 c	50 d	0 a
B. napus	1300 b	1466 ab	1416 b	1216 a	0 a
B. juncea	0 c	0 c	483 d	0 d	0 a
S. alba	1350 b	1350 b	1916 a	617 c	0 a
G. max	1566 a	1583 a	1516 b	1033 b	0 a
Pasteurized	0 c	0 c	0 e	0 d	0 a

[a] Orchard designations: WVC, Wenatchee Valley College Orchard, East Wenatchee, WA; Tukey, Tukey Horticulture Research and Experimental Orchard, Pullman, WA; and CV, Columbia View Orchard, Orondo, WA. WVC1 represents soil collected in spring 2005, and WVC2 represents soil collected in autumn 2005. CV-native soil was collected in an uncultivated area adjacent to the production orchard.
[b] Means in the same column followed by the same letter are not significantly different ($P > 0.05$; $n = 3$).

Table 3
Effect of Brassicaceous seed meal amendments on soil populations of *Pythium* spp. (cfu g^{-1} soil) recovered from non-planted experimental plots at Columbia View orchard, Orondo, WA during 2005

Treatment	*Pythium*
Control	25 b[a]
Fumigated	63 b
B. napus	675 a
B. napus-plastic	937 a
B. juncea	25 b
B. juncea-plastic	0 b
S. alba	175 b
S. alba-plastic	262 b

[a] Means in the same column followed by the same letter are not significantly different ($P > 0.05$; $n = 5$).

3.5. *Soil* Pythium *population characterization*

Total *Pythium* populations recovered from WVC2 and TU orchard soils amended with *S. alba*, *G.max*, or *B. napus* SM were significantly ($P < 0.05$) greater than in the control, pasteurized, and *B. juncea* SM-treated soils (Fig. 4a, b). In both soils, amendment with *B. napus* SM resulted in *Pythium* spp. numbers that were lower relative to *S. alba* or *G. max* SM treatment. *Pythium* species enrichment varied between the two soil types and between SMs. For example, *P. irregulare* Group I was prominent in WVC2 soil, but absent in TU soil. In contrast, TU soil amended with *S. alba*, *G. max*, or *B. napus* SM was highly enriched with *P. attrantheridium*, whereas this species was only slightly enriched by *G. max* SM amendment in WVC soil. Both soils treated with either *B. napus* or *G. max* SM were enriched with *P.* aff. *echinulatum*, whereas this species was nearly absent when soil was amended with *S. alba* SM.

4. Discussion

4.1. *Relationship between SM amendment and* Pythium *on weed suppression*

Application of Brassicaceous plant residues has been promoted as a viable strategy for the control of diverse yield-limiting pests (Lazzeri et al., 2003; Pascual et al., 2004). However, as has been the case for a variety of bio-based amendments, use of Brassicaceous residues for control of weeds and soil-borne diseases has not been widely adopted due to the inconsistency in performance realized across production systems. The ability to determine the underlying factor(s) limiting efficacy of these materials in pest control requires an understanding of the mechanism(s) leading to pest suppression. Although glucosinolate hydrolysis products, and predominantly isothiocyanates, are generally acknowledged as the primary means responsible for the biological activity of Brassicaceous plant residues, recent studies suggest that these chemistries are not the only factors responsible for the observed phytotoxic effects (Boydston and Hang, 1995; Brown and Morra, 1996; Al Khatib et al., 1997) or

Table 4
Effect of seed meal amendment on *Pythium* spp. infection of root and/or seed (%) using four different seed types in Wenatchee Valley College and Tukey Horticulture Research and Experimental orchard soils in greenhouse experiments

	T. aestivum		*V. villosa*		*A. retroflexus*		*E. crusgalli*	
	WVC2[a]	TU	WVC2	TU	WVC2	TU	WVC2	TU
Control	0 c[b]	17 c	22 c	45 b	6 cd	6 b	0 b	0 c
Control + mefenoxam	0 c	0 d	6 d	17 c	0 d	0 b	0 b	0 c
Pasteurized	11 c	0 d	0 d	0 c	0 d	0 b	0 b	0 c
Pasteurized + mefenoxam	0 c	0 d	0 d	0 c	0 d	0 b	0 b	0 c
B. napus	50 b	100 a	67 b	72 ab	28 bc	46 a	9 b	90 a
B. napus + mefenoxam	6 c	28 b	0 d	0 c	0 d	17 b	0 b	6 bc
B. juncea	17 c	0 d	28 c	68 ab	11 cd	11 b	0 b	0 c
B. juncea + mefenoxam	0 c	0 d	0 d	0 c	0 d	0 b	0 b	0 c
S. alba	87 a	100 a	94 a	91 a	80 a	62 a	60 a	0 c
S. alba + mefenoxam	6 c	0 d	0 d	6 c	0 d	6 b	0 b	11 bc
G. max	64 ab	100 a	94 a	83 a	39 b	45 a	75 a	17 b
G. max + mefenoxam	6 c	0 d	0 d	0 c	0 d	0 b	0 b	0 c

[a] Orchard designations: WVC, Wenatchee Valley College Orchard, East Wenatchee, WA; Tukey, Tukey Horticulture Research and Experimental Orchard, Pullman, WA; and CV, Columbia View Orchard, Orondo, WA. WVC2 represents soil collected in autumn 2005. CV-native soil was collected in an uncultivated area adjacent to the production orchard.
[b] Means in the same column followed by the same letter are not significantly different ($P > 0.05$; $n = 3$).

disease control (Cohen and Mazzola, 2006; Mazzola et al., 2007) attained.

Several lines of evidence from this study demonstrate that weed suppression in response to certain BSM amendments involves a microbial mechanism. These include the observation that (i) pasteurization or fumigation of soil prior to sowing of seed improved weed emergence in native soils; (ii) the application of the oomycete-selective chemistry mefenoxam reduced the weed control efficacy of most seed meal amendments; and (iii) the non-glucosinolate containing *G. max* SM provided a degree of weed control that was comparable to *S. alba* or *B. napus* SM.

The level of weed control and the effect on *Pythium* spp. populations was dependent upon the Brassicaceous species from which the seed meal was derived, and in certain instances performance in field trials relative to that obtained in greenhouse trials differed significantly. *S. alba* SM amendment resulted in the greatest and most consistent weed suppression, although field results were not always statistically significant. Lack of significance in field trials may have been the result of highly variable conditions in terms of both weed seed distribution and distribution of *Pythium* spp. in field soil environments. In contrast, amendment with *G. max* or *B. napus* SM also resulted in weed suppression, but results were not as consistent, and delayed emergence rather than plant death was sometimes observed, with seedling recovery detected during final assessment of plant emergence 21 d after seeding. Correspondingly, soil amendment with *S. alba*, *G. max* or *B. napus* SM significantly increased *Pythium* spp. populations. Soil pasteurization or treatment of SM-amended soils with mefenoxam almost uniformly increased plant emergence and biomass. *S. alba* SM-amended plots treated with mefenoxam still exhibited some reduction in plant emergence. These results support our hypothesis that plant pathogenic *Pythium* spp. mediate, at least in part, the weed suppression observed in response to BSM amendments.

Relative to other SM amendments, weed emergence data observed in response to *B. juncea* SM amendment were anomalous. *B. juncea* SM amendment did not enhance, but rather suppressed, *Pythium* spp. numbers to near or below the limit of detection, confirming its potential as an alternative treatment for the control of *Pythium* spp. (Brown and Morra, 1997; Mazzola et al., 2007). Correspondingly, this amendment did not suppress weed emergence in greenhouse trials. In certain instances, enhanced weed emergence was observed in the greenhouse in response to *B. juncea* SM, again corresponding with the negative impact of the amendment on resident *Pythium* spp. On occasion this same amendment depressed weed emergence or biomass relative to the control or control + mefenoxam treatment in field trials. It is likely that this disparity between greenhouse and field trials with regard to weed suppression resulted from the experimental design employed. In the greenhouse experiments, BSM amendments were applied 4 d

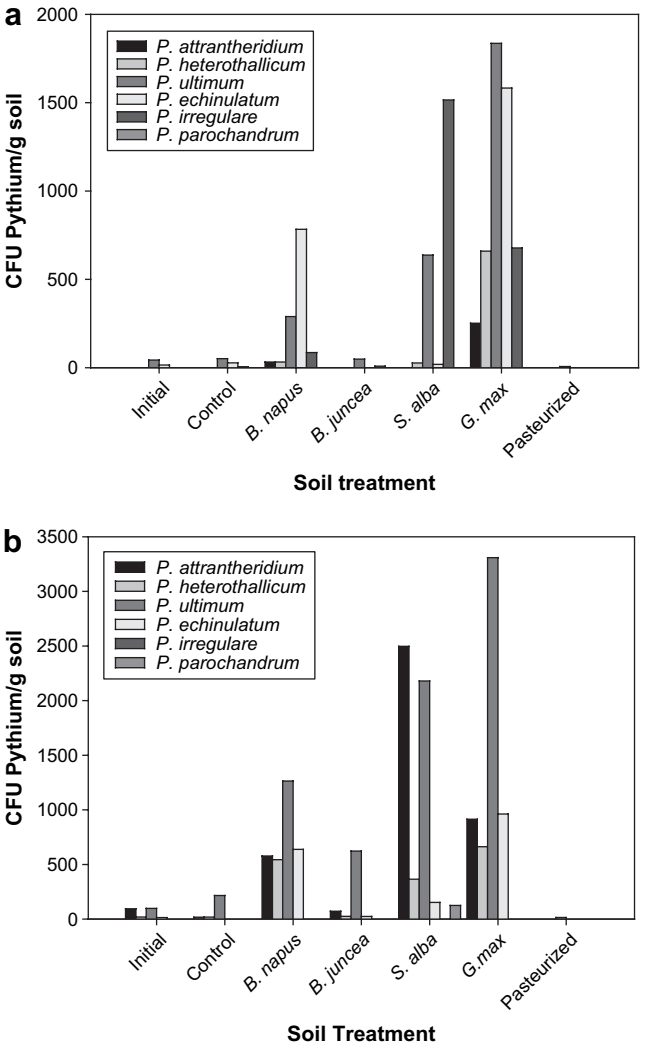

Fig. 4. Effect of seed meal amendments on *Pythium* spp. population resident to Wenatchee Valley College (a) and Tukey (b) orchard soil, as determined by real-time PCR.

prior to sowing weed seeds. As *B. juncea* SM does not stimulate *Pythium* spp. populations, the likely means of weed control observed in this study would be through generation of allylisothiocyanate (AITC). AITC emission from *B. juncea* SM-amended soils has been shown to cease within 24 h of seed meal application (Mazzola et al., 2007). Thus, it is probable that the lack of weed suppression observed in our greenhouse trials resulted from the 4-d delay in seeding of soils after *B. juncea* SM amendment, which circumvented exposure of weed seeds to AITC.

In the instance of *S. alba* SM amendment, the data indicate that multiple mechanisms contributed to the weed suppression observed in these studies. Consistent with previous research, we believe that 4-hydroxybenzyl glucosinolate hydrolysis products produced after amendment with *S. alba* SM caused injury to seeds and seedlings (Borek and Morra, 2005). However, *S. alba* SM application also resulted in elevated populations of *Pythium* spp. that caused pre- and post-emergence damping-off, which ultimately was responsible for seedling death. In contrast, weed suppression following *G. max* or *B. napus* SM amendment, with zero and low glucosinolate content, respectively, likely occurred solely in response to enrichment of and infection by resident pathogenic *Pythium* spp.

Covering *B. napus* and *B. juncea* SM-amended plots with clear plastic resulted in significantly reduced weed biomass relative to the non-treated control, a response that was not achieved in the absence of covering treated soils. This finding supports the hypothesis that weed suppression resulted in part from release of volatile hydrolysis compounds, such as AITC, derived from *p*-propenyl (allyl) glucosinolate, present to a high degree in *B. juncea* and to small extent in *B. napus* (Brown and Morra, 1997). However, application of the plastic covering could also have raised soil temperature creating optimal conditions for growth of *Pythium* spp., which exhibit greatest activity in terms of plant infection during the spring of the year (Mazzola et al., 2002). This premise is supported by the trend of increased *Pythium* spp. numbers in *B. napus* and *S. alba* SM-amended soils when covered relative to the corresponding non-covered treatments. Alternatively, it could be argued that covering the soil with plastic resulted in soil solarization, which inhibited weed emergence. However, tarping of soil occurred in May when temperatures were not high, and in a previous study conducted at this site in 2002, soil temperature under similar plastic reached a maximum of 29.4 °C in June and did not exceed 39 °C at a depth of 10 cm during July and August when annual peak temperatures occur (M. Mazzola, unpublished observations). Had temperatures been high enough (46 °C) to inhibit the seed germination of weeds, such as *Amaranthus* spp. and *E. crusgalli* (Stapleton et al., 2000), resident to this site, a corresponding reduction in *Pythium* spp. activity also would have been observed.

B. napus SM amendment and 1,3-dichloropropene-chloropicrin fumigation treatments increased weed biomass in some cases. Enhanced availability of nitrogen associated with *B. napus* SM (Snyder et al., 2006) amendment and the lower enrichment of specific pathogenic *Pythium* spp. relative to other SM as observed using real-time PCR analysis likely contributed to this outcome. Greater weed biomass in response to 1,3-dichloropropene-chloropicrin fumigation was likely due to control of resident *Pythium* spp. and reduced competition from soil microorganisms for available nutrients. Mefenoxam application to most BSM-amended and control plots either stimulated weed emergence or resulted in an increase in weed biomass relative to the control. Again, these data support our hypothesis that the enrichment of resident *Pythium* spp. in response to BSM amendments plays a significant role in the observed weed suppression.

Findings from these studies demonstrate that optimal efficacy of BSMs in the control of weeds requires function of the resident soil microbial community and specifically the activity of pathogenic species of *Pythium*. Although such a mechanism has the benefit of utilizing resident soil microbial communities, dependence of weed suppression on enhancement of resident *Pythium* spp. may lead to an inconsistency in weed control, such as that reported in previous research with Brassicaceous plant residues (Brown and Morra, 1997). Quantitatively, *Pythium* spp. populations varied widely among soils and responded differentially to SM amendment. *Pythium* spp. were not initially detected in CV orchard native soil, and this community did not respond to SM amendment. In contrast, WVC and TU orchard soils showed a differential response to SM amendments given initial populations, and community enrichment also varied among the different SM amendment types.

Initial *Pythium* spp. populations were higher in TU soil, which may explain why application of *B. juncea* did not reduce *Pythium* spp. populations to near zero, as observed in CV or WVC soil. Based upon plate count estimates, amendment of all soils with *S. alba*, *B. napus* or *G. max* SM resulted in *Pythium* spp. enrichment to around 1500 cfu g^{-1}, an increase from initial populations of 20–40× in CV and WVC soil, but only 2× in TU soil. Since equivalent amounts of SM were added to each soil, this could indicate that soils attained the maximum *Pythium* spp. populations capable of being sustained by the available substrate. Alternatively, the higher clay and OM contents in TU soil may have exerted a buffering influence that limited population expansion and/or reduced the effective available substrate. Similarly, higher clay and OM contents minimize soil acidification that results from nitrification reactions, favoring bacterial rather than fungal community enrichment in high clay and OM soils (Stotzky, 1986). In addition, recovery of allelopathic phenolic compounds varies with soil type (Dalton et al., 1989), and pretreatment of soil to remove organic matter and free metal oxides has been found to decrease sorption of phenolic compounds (Cecchi et al., 2004). These different responses to SM amendment in different soils may help to explain the variability in weed suppression observed under field conditions.

4.2. Pythium *community response to seed meal amendment*

Total *Pythium* spp. population estimates were higher using real-time PCR analyses as compared to plate counts. The disagreement in these data likely resulted from plate counts that only account for live, active cells. In contrast, real-time PCR is a gene-based approach that estimates total DNA, which could include that from spores and dead cells. Interestingly, based upon real-time PCR generated data, *Pythium* spp. populations were lower in soils amended with *B. napus* SM in comparison to *S. alba* or *G. max* SM-amended soils, which may be a function of the primer sets used. The 10 original primer sets were designed and selected based upon the most prevalent pathogenic *Pythium* spp. resident in these soils (Schroeder et al., 2006).

Many *Pythium* spp. resident to agricultural soils are non-pathogenic to most plant species and can even be beneficial to plant growth (Mazzola et al., 2002). It is plausible that *B. napus* SM amendment resulted in enrichment of a variety of *Pythium* species, many of which are non-pathogenic or less virulent, and could have contributed to the lower level of weed inhibition obtained with this SM relative to *G. max* or *S. alba* SM, despite their similar effect on total *Pythium* spp. populations. In a study in which all culturable *Pythium* spp. recovered from SM-treated orchard soil were identified, the population recovered from *S. alba* SM-amended soil was composed primarily of isolates belonging to the species *P. irregulare* Group I and *P. ultimum* var. *ultimum*, whereas that recovered from *B. napus* SM-treated soil was dominated by *P. heterothallicum* (M. Mazzola, unpublished data). *P. irregulare* Group I and *P. ultimum* var. *ultimum* are generally considered to be highly virulent plant pathogens (Chamswarng and Cook, 1985; Mazzola et al., 2002) and can cause pre- and post-emergence damping-off, whereas *P. heterothallicum* is generally a less virulent pathogen of plants and does not incite significant damping-off of wheat (Chamswarng and Cook, 1985).

4.3. Weed species response to BSM amendment

An additional factor of an agro-ecosystem that may affect efficacy of BSMs is the species composition of the weed seed bank and their relative susceptibility to the biological and chemical factors contributing to weed suppression. *T. aestivum* and *A. retroflexus* were generally more susceptible to BSM treatments than were *V. villosa* and *E. crusgalli*. Liebman and Davis (2000) speculated that small weed seeds, like *A. retroflexus*, may suffer greater allelopathic susceptibility in comparison to large seeds due to their small store of nutrient and energy reserves, and a greater root length per unit mass, which increases their relative absorptive surface area. However, Haramoto and Gallandt (2005) found monocots to be more susceptible to allelochemicals than dicots, regardless of seed size. Greater nutrient and energy reserves may enable large dicot seeds to tolerate *Pythium* spp. enrichment and may explain the reduced inhibition and later recovery observed with the large *V. villosa* seeds. In addition, *V. villosa* seeds have hard coats, which may help to reduce infection by *Pythium* spp. In contrast, the relatively large *T. aestivum* seed used in our studies exhibited high susceptibility, which may result from greater sensitivity as a monocot. Differences in rooting patterns and seed exudates could also be a factor in the differential capacity of *Pythium* species to suppress individual weed species.

Finally, the capacity of a plant species to escape initial seed meal-induced suppression has the potential to lead to increased weed biomass. The function of BSM-induced weed suppression resulting from *Pythium* spp. incited pre- and post-emergence damping-off will not only be dependent upon plant susceptibility, but also the complex of *Pythium* spp. that resides in any specific soil. Virulence towards a specific plant host varies dramatically (Mazzola et al., 2002), and individual *Pythium* species highly virulent towards one plant species may not cause significant damage to another plant species (Paulitz et al., 2003). Growth of surviving plants may be enhanced as BSMs are a significant source of nitrogen and phosphorus, and have been used in crop fertilization (Kücke, 1993).

4.4. Conclusions

Findings from this study demonstrate that multiple mechanisms determine the weed control capacity of Brassicaceous residues and that the mechanisms involved may vary among plant source. In part, selective enhancement of resident pathogenic *Pythium* spp. contributes to weed control, but this is not true for all BSMs, including *B. juncea* seed meal. The fact that plant pathogens have a role in the observed weed control has apparent implications for employing such a strategy in crop production systems, and caution must be taken in the use of such materials to prevent damage to target crops.

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